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Research paper

Modulation of auditory brainstem responses by serotonin and specific serotonin receptors

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ABSTRACT

The neuromodulator serotonin is found throughout the auditory system from the cochlea to the cortex. Although effects of serotonin have been reported at the level of single neurons in many brainstem nuclei, how these effects correspond to more integrated measures of auditory processing has not been well-explored. In the present study, we aimed to characterize the effects of serotonin on far-field auditory brainstem responses (ABR) across a wide range of stimulus frequencies and intensities. Using a mouse model, we investigated the consequences of systemic serotonin depletion, as well as the selective stimulation and suppression of the 5-HT1 and 5-HT2 receptors, on ABR latency and amplitude. Stimuli included tone pips spanning four octaves presented over a forty dB range. Depletion of serotonin reduced the ABR latencies in Wave II and later waves, suggesting that serotonergic effects occur as early as the cochlear nucleus. Further, agonists and antagonists of specific serotonergic receptors had different profiles of effects on ABR latencies and amplitudes across waves and frequencies, suggestive of distinct effects of these agents on auditory processing. Finally, most serotonergic effects were more pronounced at lower ABR frequencies, suggesting larger or more directional modulation of low-frequency processing. This is the first study to describe the effects of serotonin on ABR responses across a wide range of stimulus frequencies and amplitudes, and it presents an important step in understanding how serotonergic modulation of auditory brainstem processing may contribute to modulation of auditory perception.

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1. Introduction

Evidence accumulated over the past twenty years indicates that serotonin plays an important role in modulating auditory responses with potentially significant functional consequences. Serotonin has been linked to clinical auditory disorders such as tinnitus (Noreña et al., 1999; Simpson and Davies, 2000; Caperton and Thompson, 2011) and hyperacusis (Marriage and Barnes, 1995; Attri and Nagarkar, 2010), as well as the auditory manifestations associated with non-auditory-specific disorders including migraine (Goadsby, 1998; Hamel, 2007; Panconesi, 2008; Sand et al., 2008), depression (Gopal et al., 2000; Chen et al., 2002; Kampf-Sherf et al., 2004; Gopal et al., 2005), schizophrenia (Bleich et al., 1988; Breier, 1995; Park et al., 2010), and post-traumatic stress disorder (Southwick et al., 1999; van der Kolk, 2001; Lee et al., 2005), among others. Associations between serotonergic activity and auditory responsivity have been supported in part by auditory evoked potential (AEP) measures assessing cortical function. Here, changes in endogenous serotonin levels have been linked to changes in peak component amplitudes (Ehlers et al., 1991; Manjarrez et al., 2005) and latencies (Concu et al., 1978) as well as dynamic variation in responses to stimuli across time (Johnson et al., 1998; Stevens et al., 2006) and stimulus level (Hegerl and Juckel, 1993; Juckel et al.,

Abbreviations: 5-HT, 5-hydoxytryptamine, serotonin; 8-OH-DPAT, 8-hydroxy-DPAT hydrobromide, 5-HT1A receptor agonist; ABR, auditory brainstem response; AEP, auditory evoked potential; ANOVA, analysis of variance; dB, decibel; dB SL, decibel referenced to sensation level; dB SPL, decibel referenced to sound pressure level; IC, inferior colliculus; IP, intraperitoneal; SEM, standard error of the mean; CP93129, CP93129 dihydrochloride, 5-HT1B receptor agonist; DOI, (±)-DOI hydrochloride, 5-HT2A/C receptor agonist; Ketanserin, ketanserin tartrate, 5-HT2A/C receptor antagonist; NAS-181, (2R)-2-[[[3-(4-Morpholinylmethyl)-2H-1-benzopyran-8-yl] oxy]methyl] morpholinedimethanesulfonate, 5-HT1B receptor antagonist; pCPA, 4-chloro-DL-phenylalanine methyl ester hydrochloride, endogenous serotonin depletion agent; S-WAY, (S)-WAY 100135 dihydrochloride, 5-HT1A receptor antagonist

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1997; Juckel et al., 1999; Gallinat et al., 2000; Bruder et al., 2001; Hegerl et al., 2001; Jaworska et al., 2013). These studies generally support the theory that reduced serotonergic function is associated with increased activity and responsivity in the auditory cortex. Effects of serotonin lower within the auditory pathway have also been demonstrated in numerous extra- and intracellular studies (Ebert and Ostwald, 1992; Mizutani et al., 2006; Wang and Robertson, 1997; Fitzgerald and Sanes, 1999; Faingold, 1999; Hurley and Pollak, 1999; Hall and Hurley, 2007; Miko and Sanes, 2009), indicating the likelihood that serotonergic effects begin well before the level of the cortex. Auditory brainstem responses (ABRs) can serve as a versatile tool allowing comparison of serotonergic effects across levels of analysis. Since ABRs represent the summed activity across large populations of auditory neurons, they can illustrate whether effects observed at the level of single neurons are prominent enough to influence the amplitude or synchrony of population activity. ABRs can also be used to compare the influence of serotonergic manipulation at different sites along the auditory neuraxis, a type of information often used to identify potential sites of origin for auditory phenomena (Starr and Achor, 1975; Stockard and Rossiter, 1977). Finally, ABRs can be compared between human subjects and animal models of specific auditory disorders, facilitating testing of hypotheses on the mechanistic role of serotonin in disorders like tinnitus or hyperacusis (Tziridis et al., 2015; Heeringa and van Dijk, 2014). Despite these advantages, the effects of serotonin, and particularly specific receptor pathways, on ABR remain largely unexplored.

Serotonergic fibers and varicosities are abundant in the cochlea and each level of the auditory brainstem (Steinbusch, 1981; Willard et al., 1984; Fitzpatrick et al., 1989; Klepper and Herbert, 1991; Harvey et al., 1993; Gil-Loyzaga et al., 1997; Hurley and Thompson, 2001; Thompson and Hurley, 2004; Papesh and Hurley, 2012). Though fewer in number, some physiological investigations of serotonergic effects in the brainstem support a suppressive or partially suppressive role such that application of serotonin leads to reduced spontaneous and driven firing in the cochlear nucleus (Ebert and Ostwald, 1992), the trapezoid body (Mizutani et al., 2006), the superior olivary complex (Fitzgerald and Sanes, 1999), and the inferior colliculus (IC) (Hurley and Pollak, 2001; -Wang et al., 2008). In the IC, serotonin affects first-spike latencies and interspike intervals (Hurley and Pollak, 2005; Hurley, 2006) as well as changes in frequency tuning most often resulting in reduced response areas following serotonin appliction (Hurley and Pollak, 2001; 2005; Hurley, 2006; Hall and Hurley, 2007). ABRs provide a useful tool by which to study the aggregate effects of serotonergic manipulations across a large population of cells from the auditory nerve to the IC. Thus, a main goal of the present study was to use ABRs to characterize the effects of endogenous serotonin depletion in the mouse model across a wide range of stimulus frequencies and intensities, with the prediction that low levels of endogenous serotonin should elicit larger response amplitudes and shorter response latencies compared to baseline measures. Since the densities of serotonergic fibers are reported to be greater in low-frequency regions of several brainstem nuclei (Klepper and Herbert, 1991; Hurley and Thompson, 2001; Hurley et al., 2002; Papesh and Hurley, 2012), a further prediction is that depletion of serotonin should have larger effects on low-frequency than on high-frequency responses.

Although the effects of serotonin in the auditory brainstem are often suppressive, multiple studies reveal subsets of cells showing the opposite trend of increased responsivity with serotonin application. The diversity in serotonergic effects is largely mediated by the type and distribution of serotonergic receptors present on pre- and post-synaptic cells. Of the seven serotonin receptor families, the 5-HT1 and 2 families have been well-documented throughout the auditory system, including the periphery. Predominant effects of 5-HT1A and 5-HT2A/C receptor stimulation in the IC include reduced spiking and increased firing latencies in most cells tested in the central nucleus of the IC (Hurley, 2006, 2007), and increased frequency and amplitude of GABAergic postsynaptic currents (Wang et al., 2008). In contrast, stimulation of the 5-HT1B receptor is reported to most often elicit excitatory effects such as increases in bandwidth of frequency response maps, higher firing rates, and lower thresholds of auditory neurons (Hurley, 2006; Hurley et al., 2008; Ramsey et al., 2010). Thus, a second goal of the present work is to begin an exploration of the role of multiple specific serotonergic receptors, the 5-HT1A, 1B, and 2A/C, on the modulation of ABRs. Although this is complicated by the fact that even the same type of serotonin receptor may have different effects in different brainstem nuclei, a general prediction was that manipulating different types of serotonin receptor will create different suites of effects on the ABR.

2. Methods

2.1. Subjects

Subjects in the current study were male CBA/J (Jackson Laboratories) mice. This inbred strain is frequently chosen for auditory studies due to its good hearing sensitivity maintained across the lifespan (Mikaelian and Ruben, 1965; Willott et al., 1991). A schematic of subject groups and experimental procedures is shown in Fig. 1. A total of 56 subjects were tested at an average age of 2.3 months (standard deviation of 0.6) at baseline testing. Twenty-four of these subjects underwent depletion of endogenous serotonin. Following serotonin depletion, these 24 subjects were further divided into three groups of eight, each of which received an agonist of the 5-HT1A, 1B, or 2A/C receptor. An additional 24 subjects were placed into three groups of eight with each group receiving an antagonist of either the 5-HT1A, 1B, or 2A/C receptor. Finally, the last eight subjects received saline injections but no serotonergic manipulations. This group served as a control to ensure that changes recorded after serotonin depletion were not due to the stress of injections or handling of animals. All protocols were approved by the Bloomington Institutional Animal Care and Use Committee and were carried out in accordance with EU Directive 2010/63/EU.

2.2. Serotonergic pharmaceutical agents

Depletion of endogenous serotonin was achieved by intraperitoneal injection of 4-Chloro-DL-phenylalanine methyl ester hydrochloride (pCPA; Sigma Aldrich, Cat#C3635), which inhibits the function of tryptophan hydroxylase (Koe and Weissman, 1966). Repeated application of pCPA leads to progressive decreases in endogenous serotonin levels as serotonin stores are released and metabolized, but not replenished (Dailly et al., 2006). Subjects received a dose of 150 mg/kg pCPA diluted in physiological saline and administered intraperatoneally (i.p.) once every 24 h for six days, a regimen of pCPA injections that reduces endogenous serotonin levels by approximately 80–90% in mice (Dailly et al., 2006) while minimizing impact on nonserotonergic systems (Bauer et al., 2002). Because pCPA is reported to reduce appetite (Bubenik and Pang, 1993), subjects' weight was monitored daily to ensure that any changes in appetite resulting from pCPA did not cause undue weight loss.

To explore the role of specific serotonergic receptors on ABR measures, selective agonists and antagonists of the 5-HT1A, 1B, and 2 receptors were administered to designated subject groups (Fig. 1). Agonists were administered to subjects only after serotonin